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Short Communication

Reproducible and high-speed separation of basic drugs by capillary zone electrophoresis

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ABSTRACT

The complete separation of a mixture of seventeen basic drugs of different classes was achieved with capillary zone electrophoresis in 11 min. The migration time reproducibility for individual components was between 0.5 and 1.7% relative standard deviation (R.S.D.). Peak detection was achieved by ultraviolet absorption, with peak-area reproducibility ranging from 1.5 to 6.3% R.S.D. The pH of the running buffer was critical in determining the separation of the mixture of basic drugs. The detection of most of these components in urine and plasma is also illustrated.

INTRODUCTION

Capillary zone electrophoresis (CZE) is a recently developed separation technique based on the mobility differences exhibited by different molecules in an electric field. It has many attractive features, including being a simple, fast and highly efficient technique applicable to a wide variety of analytes (for recent reviews, see refs. 1–3). For example, CZE has been used in the analysis of cations [4], drugs [5–8], vitamin [9] and also proteins and peptides [10–12].

The development of effective methods of drug separation and determination is of importance in pharmaceutical analysis as well as in the screening and quantification of drugs in body fluids. The analysis of basic drugs by micellar electrokinetic capillary chromatography (MECC), a variant of CZE [13,14], column liquid chromatography (LC) [15–17] and gas chromatography (GC) [18–20] has been described, but the simultaneous analysis of different types of basic drugs by CZE has not been extensively reported. We wish to report here a rapid and reproducible method for the separation of a mixture of seventeen basic drugs of different classes by CZE. This method offers many advantages over some of the above techniques, including simple sample and background electrolyte preparations and a very short analysis time.

EXPERIMENTAL

Equipment

CZE was carried out with a Waters (Milford, MA, USA) Quanta 4000 capillary electrophore-

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sis system. An AccuSep (Waters) uncoated silica capillary of 60 cm × 75 μm I.D. with a detector window at 7.5 cm from the outlet end was used. The UV detector was operated at 214 nm (using a zinc lamp with a 214-nm filter). The instrument polarity was positive to negative, *i.e.* the detector at the cathodic end of capillary. All data were processed by Waters VAX-based 860 Expert Ease chromatography software.

Reagents and chemicals

Most of the basic drugs were obtained from Alltech Assoc. (Deerfield, IL, USA), with the exception of acepromazine, doxapram and medazepam, which were provided by the Racing Laboratory of the Royal Hong Kong Jockey Club (Shatin, Hong Kong). Sodium dihydrogenphosphate was purchased from Nacalai Tesque (Kyoto, Japan). Reagent-grade phosphoric acid was from Riedel-de Haen (Seelze, Germany). HPLC-grade isopropanol was from Fisher Scientific (Pittsburg, PA, USA). Other solvents and chemicals used were of reagent grade or better. Distilled water was used to prepare all aqueous solutions, which were filtered through 0.45-μm PTFE syringe filters and vacuum-degassed before use. Samples of urine or plasma extracts were similarly filtered before CZE analysis.

Procedures

The phosphate buffer was prepared by titrating 0.05 *M* sodium dihydrogenphosphate with phosphoric acid to a pH value of 2.35. This was used as the running buffer. The basic drug mixture was prepared by mixing the seventeen drugs listed in Table I in the running buffer to give individual concentrations of 31–94 μg/ml. To avoid sample decomposition, the prepared drug mixture was kept at −40°C when not in use. The capillary was conditioned at the start of each day by purging with 0.5 *M* potassium hydroxide for 5 min, distilled water for 5 min, and then the phosphate buffer for 10 min. Hydrostatic loading of the sample was performed by momentarily lifting the anodic capillary end (dipped into the sample) 15 cm above the cathodic end (dipped into the collection vial). Unless otherwise indicated, a constant voltage of 22 kV was used (current of 135–145 μA) for all experiments. For the reproducibility studies, a total of eight consecutive and identical runs were performed, with 5 min of distilled water purging and then 10 min of phosphate buffer purging in between runs.

For the analysis of human urine and plasma, a 2-ml blank sample or one spiked with the seventeen basic drugs (to individual concentrations of 0.45–1.41 μg/ml) was adjusted to pH 10.5 with aqueous sodium hydroxide and extracted gently with chloroform–isopropanol (9:1). The organic extract was separated, dried under a stream of nitrogen, reconstituted in the pH 2.35 running buffer (50 μl in the case of urine and 75 μl in the case of plasma), filtered, and then subjected to the CZE analysis.

RESULTS AND DISCUSSION

The optimum conditions for the separation as described in Table I were obtained by examining the effects of buffer pH, ionic strength and applied voltage. Of these, buffer pH was found to be the most critical factor affecting the resolution. The optimum pH for the present drug mixture was 2.35. A pH change of 0.05 units could make a big difference in separation. For example, butacaine and medazepam could not be resolved at pH 2.40, while methamphetamine and procaine overlapped at pH 2.30.

The effects of buffer ionic strength and applied voltage on resolution were less critical and were not studied at length. An increase in the buffer concentration from 0.05 to 0.075 *M* gave little improvement in resolution but greatly increased the current flow (from about 140 to about 200 μA), which was considered undesirable as it might cause electrolyte out-gassing.

An increase in the applied voltage would increase the separation efficiency up to a certain maximum [21]. The optimum was found at 22 kV (about 370 V/cm) because it gave a complete separation of all the drug components in the shortest time as well as an acceptable maximum current. An increase to 25 kV reduced the overall migration time further, but also decreased the separation efficiency.

TABLE I

REPRODUCIBILITY OF MIGRATION TIME AND PEAK AREA

Conditions: running buffer, 0.05 *M* sodium dihydrogenphosphate–phosphoric acid (pH 2.35); uncoated capillary, 60 cm × 75 μm I.D.; hydrostatic loading for 10 s; 22 kV applied constant voltage, current 135–145 μA; UV detection wavelength 214 nm.

Peak No.	Drug	Concentration[a]	Migration time[b]		Peak area[b]	
			Mean (min)	R.S.D. (%)	Mean (mV s)	R.S.D. (%)
1	Methapyrilene	42	4.53	0.85	3140	2.78
2	Brompheniramine	57	4.70	0.82	9199	2.92
3	Amphetamine	55	5.41	0.73	9539	1.52
4	Methamphetamine	31	5.56	0.70	4914	1.84
5	Procaine	73	5.67	0.52	10 940	2.12
6	Tetrahydrozoline	35	5.90	0.67	11 889	1.90
7	Phenmetrazine	42	6.01	0.63	6369	2.10
8	Butacaine	52	6.52	0.53	5162	3.29
9	Medazepam	94	6.61	0.61	28 096	1.90
10	Lidocaine	42	6.81	0.57	10 084	2.80
11	Codeine	42	6.96	0.53	20 025	2.58
12	Acepromazine	42	7.13	0.51	10 478	1.96
13	Meclizine	57	7.48	0.52	15 865	4.33
14	Diazepam	30	7.71	0.56	10 369	4.31
15	Doxapram	83	8.19	0.49	24 039	2.51
16	Benzocaine	57	10.10	1.71	14 506	5.97
17	Methaqualone	52	11.16	1.45	28 420	6.33

[a] Concentration of individual component in the mixture (μg/ml).
[b] $n = 8$.

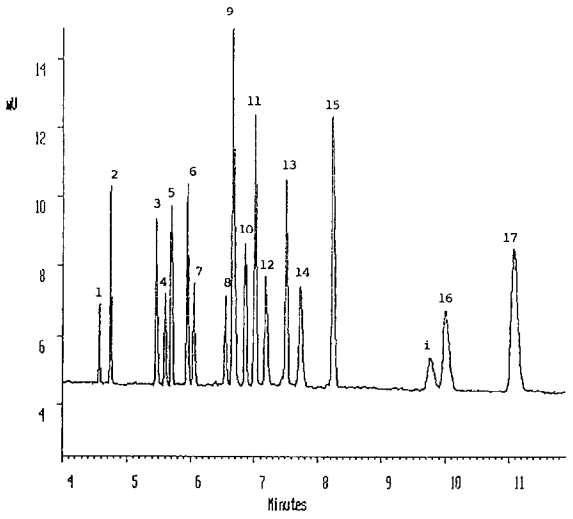

Fig. 1. Electropherogram of a mixture of seventeen basic drugs. Individual drug concentrations and other details are described in Table I. Peak i is an artifact of benzocaine (peak 16).

After optimization of the above parameters, the complete separation of the mixture of seventeen basic drugs of different classes was achieved in 11 min by CZE at pH 2.35 (Fig. 1). The pK_a values for the drugs analysed were from 2.5 to 10.5 [22]. As expected, drugs having lower pK_a values generally gave rise to longer migration times [23], although other factors such as molecular size, tendency to interact with the capillary wall and ability to form doubly charged species prevented the observation of clear correlation between pK_a and migration time. Under the present system of low pH, the electro-osmotic flow was very slow; and caffeine, essentially uncharged at this pH, had a migration time of more than 25 min (the migration time for benzaldehyde, a neutral marker, was about 28 min).

For a series of eight consecutive runs, the migration time reproducibility for individual drugs

was between 0.5 and 1.7% relative standard deviation (R.S.D.) (Table I). With the exception of the slower migrating benzocaine and methaqualone, both with a pK_a value of 2.5 [22], the values were all well below 1% R.S.D. From the same runs, the peak-area reproducibility for individual drug components was between 1.5 and 6.3% R.S.D. Again apart from benzocaine and methaqualone, R.S.D. was no more than 4.3%. The more pronounced band broadening for benzocaine and methaqualone, which presumably gave rise to less reproducible migration time and peak area, might be due to molecular diffusion or thermal effects as well as adsorption interactions as the duration of their stay in the capillary increased [21]. Band broadening in slow-migrating peaks is a general problem, and it is important to achieve separation in a short time if repeatability of migration time and peak area is of great concern. Consequently, if the present method is to be used for the analysis of other drugs with pK_a values of less than 3 (the lowest value among the fifteen faster migrating components), a more acidic pH (perhaps 1 pH unit less than the lowest pK_a) should be used. On the other hand, when optimal separation is needed for similar compounds with little difference in their pK_a values, Terabe *et al.* [24] have suggested an optimum pH of about pK_a $-\log 2$.

Weinberger and Lurie [13] separated a model mixture of eighteen drugs in 40 min using MECC at 20 kV (800 V/cm) and pH 8.5. They also analysed illicit heroin and cocaine samples. No reproducibility data were given. Nevertheless, similar analyses of acidic and neutral heroin impurities by them suggested a reproducibility problem owing to the evaporation of organic modifier from the complex MECC run buffer. For comparison purposes, the present drug mixture was also analysed by MECC: the overall voltage (20 kV) and running buffer [85 m*M* sodium dodecyl sulphate (SDS), 8.5 m*M* phosphate, 8.5 m*M* borate, 15% acetonitrile, pH 8.5) were adopted from Weinberger and Lurie [13], while the instrument, capillary and other parameters were identical to those of the present CZE method. Under these MECC conditions, the drug mixture took 25 min to elute, with two components co-eluting, and the resulting electropherogram was remarkably noisy. Increasing the overall voltage further would give even more noise, and also frequent out-gassing (conductivity failure), while decreasing it to just 18 kV would slow the run to over 40 min and still give a noisy and unresolved electropherogram.

The method described by Wernly and Thormann [14] allowed the detection of twelve basic drugs in 25 min by MECC at 20 kV (about 220 V/cm) and pH 9.1, and extended to the detection of drugs from urine extracts. However, on-column multi-wavelength detection was required to overcome the problem of incomplete drug separation, and also for the positive identification of the eluted drugs. Again, a comparative MECC analysis has been carried out in the present system, with the overall voltage (20 kV) and running buffer (75 m*M* SDS, 6 m*M* borate, 10 m*M* phosphate, pH 9.1) adopted from Wernly and Thormann [14]. The resulting electropherogram, and also others obtained at different voltages, showed little separation of the seventeen components of the drug mixture.

The present CZE method offers certain advantages over MECC, including simple background electrolyte preparation and excellent separation at very short analysis time. However, the limitation herein is the inability to analyse acidic, neutral and basic drugs together.

As an illustration of the applicability of the present method to the analysis of drugs in biological samples, extracts of human urine or plasma spiked with the same seventeen drugs were analysed, along with extracts of their corresponding blanks. The individual drug concentrations in either spiked samples were between 0.45 and 1.41 μg/ml. The resulting electropherograms are shown in Figs. 2 and 3. A matrix effect that slightly reduces the migration times of the drug components was observed when biological extracts were analysed. This effect disappeared with careful rinsing of the capillary, and migration times returned to normal in subsequent runs with the standard drug mixture. Because of the very clean urine and plasma backgrounds obtained,

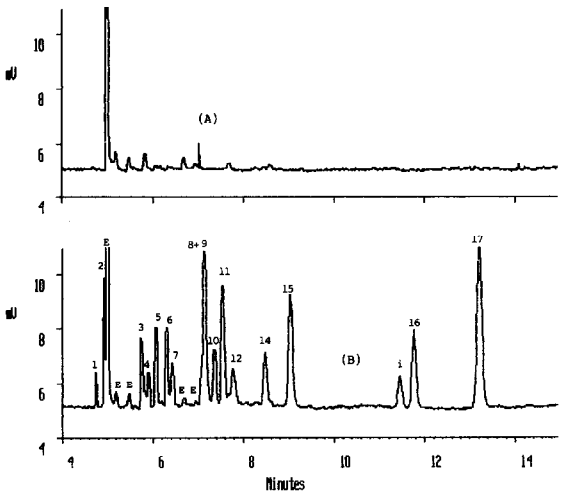

Fig. 2. Electropherograms of (A) extract of blank urine and (B) extract of urine spiked with the same mixture of seventeen basic drugs as in Fig. 1 (concentrations range from 0.45 to 1.41 μg/ml of urine). The electrophoretic conditions and peak identification are described in Table I and Fig. 1. Peaks E are endogenous components.

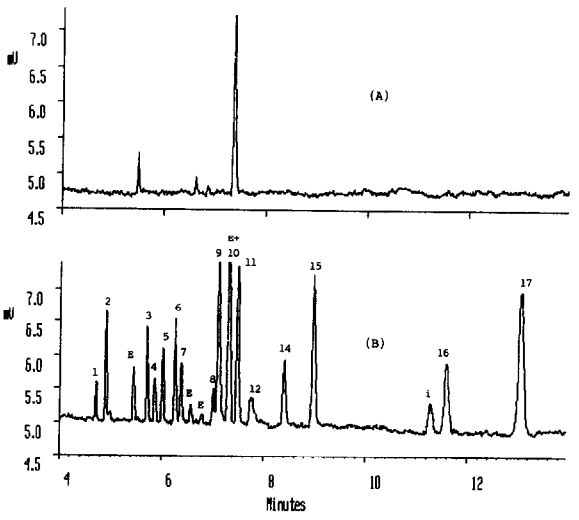

Fig. 3. Analysis of (A) extract of blank plasma and (B) extract of plasma spiked with the same mixture of seventeen basic drugs as in Fig. 1 (concentrations range from 0.45 to 1.41 μg/ml of plasma). Electrophoretic conditions and peak identification are described in Table I and Fig. 1. Peaks E are endogenous components.

most of the seventeen drugs were easily detected by the present method. Meclizine was an exception: it could not be extracted. However the drug could easily be identified by the direct analysis of filtered urine spiked at about 10 μg/ml.

CONCLUSION

The present findings demonstrate the power of CZE in a simple, fast and very efficient separation of a wide variety of basic drugs at low pH. The reproducibility and other data suggest its broad applicability to the simultaneous determination of drug components in pharmaceuticals, and also to the detection and quantification of basic drugs in biological specimens.

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REFERENCES

1 Z. Deyl and R. Struzinsky, *J. Chromatogr.*, 569 (1991) 63.
2 C. C. Campos and C. F. Simpson, *J. Chromatogr. Sci.*, 30 (1992) 53.
3 W. G. Kuhr and C. A. Monnig, *Anal. Chem.*, 64 (1992) 389R.
4 A. Weston, P. R. Brown, P. Jandik, W. R. Jones and A. L. Heckenberg, *J. Chromatogr.*, 593 (1992) 289.
5 K. Saloman, D. S. Burgi and J. C. Helmer, *J. Chromatogr.*, 549 (1991) 375.
6 U. Seitz, P. J. Oefner, S. Nathakarnkitkool, M. Popp and G. K. Bonn, *Electrophoresis*, 13 (1992) 35.
7 M. T. Ackermans, J. L. Beckers, F. M. Everaerts, H. Hoogland and M. J. H. Tomassen, *J. Chromatogr.*, 596 (1992) 101.
8 S. K. Yeo, H. K. Lee and S. F. Y. Li, *J. Chromatogr.*, 585 (1991) 133.
9 E. Kenndler, C. Schwer and D. Kaniansky, *J. Chromatogr.*, 508 (1990) 203.
10 K. A. Cobb and M. V. Novotny, *Anal. Chem.*, 64 (1992) 879.
11 F. Foret, S. Fanali and P. Bocek, *J. Chromatogr.*, 516 (1990) 219.
12 P. D. Grossman, J. C. Colburn, H. H. Lauer, R. G. Nielsen, R. M. Riggin, G. S. Sittampalam and E. C. Rickard, *Anal. Chem.*, 61 (1989) 1186.
13 R. Weinberger and I. S. Lurie, *Anal. Chem.*, 63 (1991) 823.
14 P. Wernly and W. Thormann, *Anal. Chem.*, 63 (1991) 2878.
15 A.Turcant, A. Premel-Cabic, A. Cailleux and P. Allain, *Clin. Chem.*, 37 (1991) 1210.
16 P. Linares, M. C. Gutierrez, F. Lazaro, M. D. Luque De Castro and M. Valcarel, *J. Chromatogr.*, 558 (1991) 147.

17 P. R. Puopolo, S. A. Volpicelli, D. M. Johnson and J. C. Flood, *Clin. Chem.*, 37 (1991) 2124.
18 D. Fretthold, P. Jones, G. Sebrosky and I. Sunshine, *J. Anal. Toxicol.*, 10 (1986) 10.
19 C. G. Georgakopoulos, J. C. Kiburis and P. C. Jurs, *Anal. Chem.*, 63 (1991) 2021.
20 G. P. Neill, N. W. Davies and S. McLean, *J. Chromatogr.*, 565 (1991) 207.
21 J. Liu, V. Dolnik, Y.-Z. Hsieh and M. Novotny, *Anal. Chem.*, 64 (1992) 1328.
22 C. J. Drayton (Editor), *Comprehensive Medicinal Chemistry*, Vol. 6. Pergamon Press, Oxford, 1990.
23 M. Mizukami, N. Nimura, T. Kinoshita, T. Hanai and H. Hatano, *J. High Resolut. Chromatogr.*, 14 (1991) 561.
24 S. Terabe, T. Yashima, N. Tanaka and M. Araki, *Anal. Chem.*, 60 (1988) 1673.